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Abstracts

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V: Plenarvorträge
A: Abstract
A1: Wissenschaftspreis
A2–7: Abstract I Transplantation
A8–11: Abstract II Immuntherapie, Nicht-klassische HLA-Merkmale, Granulozytenantigene
A12–17: Abstract III Methoden, Genetik, Krankheitsassoziation
P: Poster
P18–29: Poster I Methoden, Genetik, Krankheitsassoziation
P30–36: Poster II Transplantation, Immuntherapie, Nicht-klassische HLA-Merkmale, Granulozytenantigene
M: MTA-Workshop
M1–7

V: Plenarvorträge

V1

HLA Class I Molecules and Its Relatives: Structural Diversity and Dynamic of Ligand Recognition

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Major histocompatibility complex (MHC) HLA class I molecules bind and present short peptides derived from self and non-self proteins to T cells as part of the immune response. During recent years the three-dimensional structure of many class I variants in complex with a variety of peptides has been determined by X-ray crystallography. In addition, the structure of class I molecules in complex with T-cell receptors and several proteins that modulate the immune response was solved. Many class I homologs have been discovered. These homologs often adopt a three-dimensional structure very similar to the class I fold but may have a different function.

In addition to the structure of class I molecules, an understanding of the conformational dynamics is essential for an understanding its function. Computer simulation methods play an increasing role to better understand the molecular dynamics of proteins and its role during peptide recognition and interaction with other proteins. An overview on available structural data on class I molecules and related molecules will be given. The application of computer simulation methods in particular molecular dynamics methods to study peptide recognition by class I molecules and how it can be used to interpret experimental data will be discussed.

V2

HLA Functions in Nature and Laboratory

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HLA molecules are peptide receptors. Although their central function includes peptide presentation to T cells, MHC molecules have been discovered by an artificial function, which is graft rejection caused by alloreactive T cells. This artefact stll has a major impact on transplantation, but graft rejection does not belong to the genuine functions of HLA molecules. By their presented peptides, HLA molecules play a central role in the adaptive immune system and exert a variety of functions, which include selection of T cells in the thymus, inhibition of NK cells, and activation or tolerization of T cells in lymphoid organs or in the periphery. As a special effect in transplantation immunology, minor H antigens, which are polymorphic peptides displayed by HLA leading to canonical T cell responses, contribute to graft rejection.

HLA-presented peptides are generated by antigen processing, a multi-step procedure much of whose details are well understood nowadays. The knowledge of many of the features of antigen processing has lead to a large set of prediction programs that forecast the presentation of peptides by HLA molecules. In addition to our classical program SYFPEITHI, we have recently developed a novel software tool for the prediction of minor H antigens, SNEP.

HLA functions can, however, not only be utilized by in silico work. Recombinant HLA molecules have been available for several years and can be used for a variety of different purposes. HLA-tetrameric constructs are used in T cell assays, whereas HLA-coated microspheres efficiently prime T cells in vitro. In summary, HLA properties are exploited for many different diagnostic and experimental applications.

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V4

Gefühle, Gerüche, Gene: Partnerwahl und MHC

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Polymorphe Gene des *MHC* und Loci für olfaktorische Rezeptoren (OR) sind wiederholt mit verschiedenen Aspekten von Partnerwahl und reproduktivem Erfolg in Verbindung gebracht worden. Im Gegensatz zu *MHC* homozygoten Individuen sollten solche mit *MHC* Heterozygotie einen Vorteil bei der Bekämpfung von Krankheitserregern aufweisen. Eine geruchsbeeinflußte Partnerwahl mag primär der Sicherstellung der *MHC* Heterozygotie bei den Nachkommen dienen. Selektive Prozesse zur Sicherung eines optimalen reproduktiven Erfolges könnten auch bei Wirbeltieren nicht nur vor der Kopulation existieren, sondern auch postkopulatorisch („cryptic female choice"). Gemeinsam mit *MHC*-kodierten Molekülen könnten die Produkte von *OR* Genen prinzipiell beteiligt sein, etwa bei der Hinleitung von Spermien zur Eizelle. Zusammen mit früher erhobenen Befunden zur Expression von *HLA* Klasse I schweren Ketten in Spermienvorläuferzellen könnte die Expression von *OR* im Hoden auf eine funktionelle Verbindung zwischen polymorphen Molekülen des *MHC* und OR hindeuten. Falls nur OR zur Expression auf Spermien gelängen, welche nicht in der Lage sind, mit eigenen („selbst") Molekülen zu interagieren, könnten sie von Molekülen (z. B. hochpolymorphen MHC Molekülen oder ihren Fragmenten) im weiblichen Genitaltrakt angelockt werden, die ihnen als „nicht-selbst", also fremd, erscheinen. Diese Form des „cryptic female choice" könnte bereits in Kraft treten, bevor Spermium und Eizelle aufeinander treffen, etwa durch die Etablierung chemischer Gradienten im Eileiter. Auf diese Weise würden bevorzugt diejenigen Spermien in die Nähe der Eizelle gelangen, die mittels ihrer OR bestimmte Moleküle des weiblichen Organismus als „nicht-selbst" erkennen. So könnte die Wahrscheinlichkeit eingeschränkt werden, daß die Eizelle von einem genetisch ähnlichen Spermium befruchtet wird. Eine kostspielige Investition in einen *MHC* homozygoten Embryo mit möglicherweise suboptimalen genetischen und immunologischen Eigenschaften könnte auf diese Weise vermieden werden.

V5

Deficiency of Mannose Binding Lectin and Susceptibility to Infectious Diseases

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Genetic polymorphisms within the innate immune system are increasingly being identified and their role in a clinical setting is under investigation. The best-understood and investigated defect is Mannose-binding lectin (MBL) deficiency. MBL, a secreted pattern recognition receptor binds to a wide range of clinically relevant microorganisms and activates the complement system via an antibody independent pathway.

Human deficiency of MBL was first identified in 1989 in association with a common defect of opsonisation in children and subsequent studies have confirmed that MBL deficiency not only predisposes to infectious illness but also modulates disease progression (eg HIV, rheumatoid arthritis and cystic fibrosis). Human deficiency of MBL is predominantly caused by point mutations within exon 1 of the MBL gene at codons 52, 54, or 57, which result in aminoacid substitutions that compromise assembly of functional oligomers. Individuals heterozygous for these mutations have reduced concentrations of MBL in serum, whereas the protein is almost absent from the serum of homozygotes and compound heterozygotes. In addition to the exon 1 mutations there are three major polymorphisms in the promotor region of the MBL gene, and one of these variants (X/Y) also profoundly influences expression of the protein. More than a third of the population will have haplotypes that predispose to low MBL concentrations, and very low concentrations are found in approximately12%. MBL deficiency is thought to be clinically most apparent in the context of co-existing immune defects, including primary and secondary immune deficiencies. Patients at risk with a low MBL status could benefit from MBL replacement therapy.

V6

Non-HLA Genetic Risk Factors in Allogeneic Stem Cell Transplantation

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On behalf of the Eurobank members (EC grant QLRT-2000-00010)

The pathophysiology of Graft-versus-Host Disease (GvHD) and complications following allogeneic stem cell transplantation results from a complex interaction of innate and adaptive immunity involving cellular effectors as well as cytokines. As on a functional level patients show extremely heterogenous inflammatory patterns in spite of identical treatment we assumed a major role of genetic polymorphism in immunoregulation following allogeneic SCT: We therefore analysed a variety of cytokine gene single nucleotide polymorphisms (SNPs) in a cohort of more then 400 HLA-identical sibling transplants from 6 European centres. In addition, we addressed the role of activation of the innate immune system by analysing SNPs within the NOD2/CARD15 gene in more than 600 related and unrelated transplants. The NOD2/CARD15 molecule serves as an intracytoplasmatic pattern recognition molecule for bacterial cell wall compounds, and SNPs within the gene have been shown to be genetic risk factors for Crohn´s disease. DNA from recipients and donors was analysed using specific PCRs, and results were compared with major outcome variables such as severe GvHD (grade III/IV), non-relapse mortality and overall survival.

Specific cytokine gene SNP alleles observed within donors or recipients such as "TNFd3,3", "IFNgamma 3,3", "IL6 GG" revealed some significant associations with severe GvHD, and especially presence of "IFNgamma 3,3" translated in improved overall survival even in multivariate analysis. The strongest associations so far, have been observed for variants within the NOD2/CARD15 gene, as presence of variants either in recipients or in both, recipients and donors was predictive for increased GvHD and treatment related mortality and worse overall survival in HLA-identical sibling transplants which was significant in 2 consecutive cohorts of patients even in multivariate analysis.

The major challenge of the next years is to integrate these diagnostic and prognostic parameters into clinical care of patients after confirmation by carefully planned prospective trials: They hopefully will be used to develop risk indices with regard to stem cell transplantation related complications which then can be applied to modify either prophylactic or therapeutic strategies. In addition, the observed association may result in new insights in the pathophysiology of GvHD and transplant related complications: Thus, the association of NOD2/CARD15 variants with GvHD and mortality could help to explain the long standing association between intestinal bacteria and GvHD, as altered behaviour of these receptors of bacterial cell wall compounds results directly in increased NFkB related inflammation.

V11

Immune System and Cytomegalovirus – a Race between the Hare and the Hedgehog?

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The human cytomegalovirus ("hedgehog") is a member of the herpes virus family and has been one of the most faithful companions of mankind ("hare") for many million years.

If the human cytomegalovirus (HCMV) is not active during a (re)infection, it "sleeps" in a latent status. However, in immunocompromized patients this "harmless" species may turn into a severe aggressor. The prevalence of HCMV in different human populations varies strongly (50% to nearly 100%). The dsDNA genome of the HCMV covers more than 230,000 bp, which code for more than 200 proteins.

HCMV has developed a multiplicity of most different strategies to modify the human immune system (e.g., viral immune evasion, immunomodulation). Particularly the HLA class I expression on the cell surface serves as a target structure. (Cells lacking HLA class I molecules on their surfaces are usually attacked by NK cells.) The virus genome codes also for a HLA class I similar protein, which is called UL18 (unique long region) because of the gene localization. This protein can inhibit NK cells by binding to their LIR-1 (leukocyte immunoglobulin-like receptor). In the meantime it has become clear that certain cytotoxic T-cells do precisely recognize UL18 over LIR-1 and consequently eliminate HCMV infected cells.

Although HCMV does not belong to the oncogeneic viruses in a narrower sense, evidence is growing that HCMV may influence the emergence, development, recovery and predisposition for relapse of many oncological diseases (oncomodulation).

Let us keep our fingers crossed that our immune system keeps on nosing out HCMV in this race.

V13

The Molecular Basis of T Cell Receptor Gene Therapy of Malignant Disease

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Circumventing antigen-specific self-tolerance in HLA-A*0201 (A2.1) transgenic mice allows the generation of A2.1-restricted T cell antigen receptors (TCR) with high affnity for universal human tumor- and leukemia-associated peptide epitopes (TAA) (i. e., MDM2, p53). Retroviral transduction of human T lympocytes with wild-type and partially humanized mouse-derived TCR leads to the equipment of the otherwise tolerant T cell repertoire with effective cytotoxic T lymphocytes (CTL) with high avidity for a wide range of A2.1-positive tumors and leukemias. These results emphasize the successful preclinical establishment of TCR gene transfer as a novel molecular concept for the immunotherapy of malignant disease.

However, implementing TCR-based gene therapy of malignant disease requires further molecular optimization and research. To prevent or impair potentially harmful recombination of transduced and endogenous TCR chains, we have modified the interface of TCR a and b chains. Designing various single chain TCR constructs met the same purpose.

Developing CD8-independent TCR allowed not only the reprograming of $CD4^+$ T helper cells (Th), but also turned human $CD8^+$ CTL into hyper-avidity effector cells.

As the death versus survival fate of TCR transduced T cells is coregulated by conserved TCR transmembrane sequences, specific alterations within these TCR domains could create effector CTL equipped with beneficial properties for cancer immunotherapy, such as an increased proliferative capacity and prolonged survival.

A: Abstract

A1

HLA-A1: Peptide-Restricted Antibody Binding Induces Major Conformational Changes of HLA-A1 Heavy Chain Residues

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Human leukocyte antigen (HLA) class I molecules are heterotrimeric complexes consisting of a heavy chain (HC), β2-microglobulin and a peptide, and are located on the surface of nucleated cells. HLA- A1 complexed with a MAGE-A1-derived peptide (EADPTGHSY) occurs only on certain tumour cells and is a promising target for cancer immunotherapy. We have previously determined the structure of HLA-A1:MAGE-A1 complexed with an affinity-matured recombinant antibody fragment, Fab-Hyb3 (J. Biol. Chem. 280: 2972 – 2980, 2005). Here we present the structure of HLA-A1:MAGE-A1 and compare it with that of HLA-A1:MAGE-A1:Fab-Hyb3 in order to gain insight into possible structural changes induced by the binding of the antibody fragment. The complex was prepared from the peptide and recombinantly expressed protein chains, followed by crystallization. One crystal diffracted to 1.8 Å resolution. The collected X-ray data was used for structure determination and the phase problem was solved by molecular replacement. Only marginal differences in the conformation of the peptide in the two structures were observed, but Fab-Hyb3 binding resulted in major rearrangements of heavy chain residues Arg65, Gln72, and Arg145. These amino acids are contacted by residues belonging to the complementarity-determining regions (CDR) 1 and 3 of the heavy chain and CDR3 of the light chain of Fab-Hyb3. Our results indicate that the specificity and high affinity of Fab-Hyb3 in binding to its target is associated with antibody-induced repositioning of side chains of residues within the α1-helix and the N-terminal part of the α2-helix of the HLA-A1 molecule.

A2

Is the Pre-Transplant Serum Level of sCD30 Really a Potential Risk Factor for Kidney Transplant Rejection?

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There exist recent data which suggest that the pre-transplant level as well as changes in the post-transplant levels of soluble CD30 molecule (sCD30) may be a risk for acute rejection and a worse prognosis of the transplanted cadaver kidneys. Soluble CD30 is known as an activation marker for the T helper 2 type-mediated immune response. It was the aim of this first preliminary study to investigate the serum concentrations of sCD 30 of the complete waiting list of kidney recipients of the DSO region „East (n = 982) comprising four German transplant centres. For its determination an ELISA assay commercially available (Biotest AG, Dreieich, Germany) was used. Unexpectedly, our data are not in accordance with the previous data (n = 3899) of that study performed by Süsal et al. (2002) with samples from 29 transplant centres from Western Europe (n = 2747), South-Eastern Europe (n = 380), North America (n = 566), South America (n = 77) and Australia (n = 129). The following table summarizes our data on the sCD30-concentrations of 982 recipients (Middle-European Caucasians) from April 2005:

sCD30 (conc.)	less than 100 U/ml	100-300 U/ml	more than 300 U/ml
anti-HLA Ab pos.	144	25	1
anti-HLA Ab neg.	782	27	3
samples per group	926	52	4
percent per group	94.3	5.3	0.4

Our data onfirm that the serum levels of sCD30 are independent from the pre-transplant anti-HLA antibody (Ab) level. However, we did not detect the high percentage of recipients showing a sCD30-level higher than 100 U/ml as described previously.

A3

Verlaufskontrollen von sCD30 bei Patienten vor Nierentransplantationen (NTX)

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Ein prätransplantär erhöhter Serum-Spiegel an löslichem (s)CD30 kann ein Risikofaktor für eine spätere Abstoßung einer transplantierten Niere sein. Ziel der Arbeit war die Erhebung von Verlaufskontrollen der sCD30-Werte an Patienten auf der NTX-Warteliste und die Definition einer sinnvollen Frequenz der prätransplantären sCD30-Testung.
Hierfür wurden 90 Patienten vor NTX im Rahmen des HLA Antikörper-Screenings über drei Quartale mittels sCD30 Instant ELISA (Bender Med. Austria) auf sCD30-Werte im Serum untersucht.
Bei 6 der 90 Patienten fanden sich in allen drei Quartalen erhöhte sCD30-Werte (> 100U/ml) mit einem Mittelwert von 141U/ml (Maximum: 248,5U/ml). 20 Patienten zeigten mindestens einmal einen Wert über 100U/ml (MW 133,1U/ml). Der Mittelwert der im Normbereich gelegenen sCD30-Werte lag bei 50,88U/ml.
Die Unterschiede der sCD30-Werte zwischen zwei Quartalen lagen zwischen 0,1 und 116,2 U/ml, wobei ein Großteil (79,4%, n = 146) weniger als 30U/ml betrugen. Bei 18.4% lag die Differenz zum Vorwert zwischen 30 und 100U/ml (n = 30). Bei 2,2% der Messungen (n = 4) betrug die Abweichung mehr als 100U/ml. Ein signifikanter Zusammenhang zwischen sCD30-Spiegel und vorsensibilisierten bzw. bereits transplantierten Patienten konnte vorerst nicht gefunden werden. Die sCD30-Werte bei nichtsensibilisierten Patienten ergaben im Mittel 57,6U/ml, bei Patienten mit einer PRA > 5% 69,2 U/ml und bei Vortransplantierten 66,2U/ml.
Aufgrund der erhobenen Schwankungen der sCD30-Werte erscheint eine unmittelbare prätransplantäre Bestimmung sinnvoll. Ist dies im Routinebetrieb des Labors nicht möglich, stellt die Einbindung der Bestimmung in das quartalsmäßige Antikörperscreening eine Alternative dar.

A4

DNA-Chimerism in Cell-Free Urine after Renal Transplantation

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Background: New non-invasive screening approaches for the diagnosis of acute rejection (aRx) after renal transplantation might avoid potential biopsy complications. DNA-chimerism can be detected in cell-free urine of kidney transplant recipients and is a potential source of material for diagnosis. We hypothesized that donor-derived DNA in the recipients cell-free urine is a phenomenon associated with aRx.
Methods: 232 urine samples from 56 HLA-mismatched kidney transplanted patients were collected during hospitalization and after release from the hospital. Genomic DNA was prepared and donor-derived DNA was measured by real-time PCR using sequence-specific primers defining serological HLA-specificities. 35 patients developed aRx whereas 21 patients with stable renal function served as controls. Levels of donor-derived DNA normalized to a housekeeping gene in 79 samples from control patients were compared to the expression in 153 samples from patients with aRx (Mann-Whitney-U-Test).
Results: Interestingly, the analysis revealed that in 194 of the investigated 232 urine samples free donor-derived DNA was present. In contrast to our hypothesis the levels of urinary donor-derived DNA in patients with aRx were not significantly higher than in patients with stable renal function (p=0.971).
Conclusions: Our study supports the finding of Zhang et al. (Clin Chem. 1999) that donor-derived DNA in cell-free urine is detectable after kidney transplantation. But our analyses of extended data indicate that there might be no relationship between DNA-chimerism and aRx episodes.

A5

The Induction of the Activating NK Cell Receptor NKG2D mRNA Is Associated with Acute Rejection Following Kidney Transplanation

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Introduction: NKG2D is an activating natural cytotoxicity receptor which binds to MHC class I chain-related (MICA) antigens. The stress-inducible MICA molecules have been shown to be expressed on e.g. epithelial, endothelial cells and activated $CD4^+/CD8^+$ T cells. Acute rejection (aRx) following renal transplantation implicates cellular stress within the graft. To assess whether an induction of MIC antigen leads to enhanced activation of NKG2D during aRx, we examined mRNA profiles of both markers in biopsies and urine sediment during aRx.
Material and Methods: Using RT-PCR 30 biopsy samples diagnosed as aRx were investigated for MICA and NKG2D mRNA expression and were compared to control biopsies (n=32). In a second patient group 187 urine samples were collected from renal-allograft rejecting recipients (n=56) during aRx and compared to 43 patients with stable renal function (n=84).
Results: The analysis revealed a significant mRNA upregulation of NKG2D ($p<0.001$) in biopsies diagnosed as aRx compared to the controls. ROC curve analysis for NKG2D demonstrated a sensitivity and specificity of 83% and 78%. High mRNA induction particularly for the NK cell receptor NKG2D ($p<0.01$) was uncovered in urine sediment in patients with aRx. However, no significant mRNA induction for MICA was observed for biopsy and urine specimens.
Conclusion: Our data describe the significant mRNA expression of NKG2D during renal aRx suggesting the triggering of innate NK cell responses during renal allograft aRx.

A6

Mismatches of HLA Class I Alleles in Haematopoietic Stem Cell Transplants

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Matching of unrelated donors (URD) and patients for HLA class II alleles favours the outcome of haematopoietic stem cell transplantation (HSCT). The significance of HLA class I allelic mismatches for transplant outcome is under discussion. Reports on long-term effects like chronic graft-versus-host disease (GVHD) are rare. We studied the association of human leukocyte antigen (HLA) class I allele mismatches and outcome in 144 patients given HSCT from URD who were matched for HLA-DRB1, DRB3/4/5, and DQB1 alleles. The risk of chronic GVHD was significantly increased in patients with class I mismatched donors, the mismatch either detected by low- or high-resolution typing, i.e. an antigenic or allelic mismatch significantly increased the risk of chronic GVHD. Overall survival was significantly reduced in patient/donor pairs with more than one-allele class I mismatch. Thus, selection of unrelated donors for transplantation should be based on high-resolution HLA class I typing.

A7

Langzeitbeobachtung der zellulären In-vitro-Immunität nach allogener peripherer Blutstammzell- vs. Knochenmarktransplantation

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Innerhalb der ersten elf Monate wurde nach allogener peripherer Blutstammzelltransplantation (PBSCT) vs. Knochenmarktransplantation (KMT) eine verbesserte Immunrekonstitution beschrieben (Ottinger et al. Blood 1996; 7:2775). Methodisch kam hierbei der Lymphozytentransformationstest (LTT) zum Einsatz, bei dem mononukleäre Zellen der Patienten mit vier Mitogenen und zwölf Recall-Antigenen stimuliert wurden. Eine Analyse von LTT-Daten >15 Monate nach allogener PBSCT ist aber bisher nicht publiziert. In diese Studie wurden 408 Patienten nach PBSCT und 324 nach KMT eingeschlossen, von denen insgesamt 2018 Proben bis 20 Jahre nach Transplantation mittels LTT untersucht wurden. Die Daten initial nach Transplantation konnten (als Trend) bestätigt werden, die Verläufe im 2. und 3. Jahr waren in beiden Gruppen vergleichbar, dann aber zeigte sich ab dem 4. Jahr nach Transplantation in der KMT-Gruppe eine signifikant stärkere Reaktionen auf Mitogene und Recall-Antigene ($P<0,05$ im 4. bzw. $P<0,01$ im 4. und 6. Jahr). Die Unterschiede in der Antigenreagibilität waren durch eine erhöhte LTT-Reaktion auf Herpesviren (HSV-Typ 1 und VZV) und Influenza A und B Virus bedingt. Ferner war HLA-DRB1*0701 mit einer signifikant schlechteren Reaktion auf HSV-Typ 1 assoziiert ($P<0,05$). Die Antigenreaktion erreichte in beiden Gruppen etwa 2 1/2 Jahre nach Transplantation das Niveau von Kontrollen (n=374). Damit zeigt sich im ersten Jahr nach Transplantation, in dem eine besondere Infektionsgefahr besteht, ein Benefit in der PBSCT-Gruppe, dieser ist aber ab dem zweiten Jahr nicht mehr nachweisbar.

A8

In Silico Approach to Identify GvL-Relevant MHAGs: Expression Profiling, HLA Peptide Binding and Processing Prediction of SNP Areas

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Minor histocompatibility antigens (mHags) encoded by coding single nucleotide polymorphisms (SNPs) are capable of inducing allogeneic T cell responses in HLA-identical transplants. SNP-caused amino acid variations can result in a differential HLA peptide binding or proteasomal processing and thus significantly influence mHag presentation. To identify potential graft versus leukemia mHag sources, an array-based gene expression profiling in hematopoietic and cells of graft versus host disease target organs was performed to single out hematopoietically expressed proteins. Subtraction profiling showed that 388 genes were expressed selectively in both CML and CD34 cells but not in keratinocytes or colon epithelial cells. In 102/388 genes, 220 allele-frequency typed SNPs were found. For 151 SNPs, 310 mono- or biallelic epitopes were concordantly predicted by two different HLA binding prediction algorithms. Predicted epitopes were analyzed for proteasomal processing by three different algorithms using stringent criteria. The constitutive proteasome algorithm identified 4/30 C-terminally processed epitopes not destroyed by any internal cleavages. The immunoproteasomal algorithm predicted 8/40 epitopes without internal cleavages. Only 6 epitopes were predicted to be processed by both types of proteasomes. It is expected that genome-wide expression and SNP profiling combined with HLA peptide binding and proteasomal processing prediction will be an effective tool in identifying new mHags sources.

A9

HLA-B*4103 Preferentially Binds Peptides of Extraordinary Length

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As B*4103 has been identified to provide extremely valuable peptide binding data for characterizing peptide motifs for numerous other HLA-B alleles, recombinant B*4103 molecules were generated and eluted peptides determined by mass spectrometry. The length of the identified peptides ranged between 10-15 amino acids (AA). More than half of the peptides exceeded 11 AA, suggesting that these peptides have the tendency to bulge out of the peptide binding region (PBR) to compensate for length. For anchor position P2 of the ligands, a striking preference for Glu (polar, acidic) could be observed, while the omega position showed a preference for Phe and Leu (neutral, hydrophobic). Among pockets A, B, D, and F, B*4103 shares identical pocket sequences with a total of 28 other HLA-B alleles. In this regard the B*4103 peptide binding data contribute substantially to the modular concept for peptide binding prediction by considering HLA molecules as mosaics to transfer peptide binding predictions to multiple other HLA alleles. The P2/P omega binding motif (Glu at P2 and Leu at P omega) of B*4103 is also displayed by the B*40 alleles, which possess an almost identical AA composition of pockets B and F when compared to B*4103. As peptide motif similarities between different alleles have been suggested to be considered for donor selection when no HLA identical donor is available, the tendency of certain alleles to present longer peptides should be taken into account. Despite identical motifs the preference for longer peptides bulging out of the PBR are predictive for a strong allogeneic T cell response.

A10

Comparative Structural and Thermodynamic Studies of HLA-B*2703 and Other HLA-B27 Subtypes Complexed with a Viral and a Self-Peptide

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The human class I allele *HLA-B27* is strongly associated with ankylosing spondylitis (AS). The HLA-B*2703 subtype is restricted to black individuals, but exhibits a questionable association with AS. The B*2703 molecule differs from the prototypical subtype *B*2705* only at the heavy chain (HC) residue 59 where Tyr is substituted by His. This exchange is expected to affect the anchoring of the N-terminal peptide residue within the A-pocket of the molecule. The peptide N-terminus is typically fixed by a pentagonal hydrogen bonding network. We investigate the influence of HC polymorphism on structural and thermodynamic properties of HLA-B27 subtypes and compare here B*2703 complexed with a viral (pLMP2, RRRWRRLTV) and a self-peptide (pVIPR, RRKWRRWHL) to B*2705 and B*2709 (*J.Exp. Med.,* 2004; *J. Biol. Chem.,* 2005). The structures of B*2703:pLMP2 and B*2703:pVIPR were solved by X-ray diffraction. Both peptides are bound to the groove in drastically different modes, resembling the structure found in B*2709:pLMP2 and one of the two conformations found for B*2705:pVIPR. The conformation of the peptide N terminus is not influenced by the lack of a hydroxyl group in His59 if compared to Tyr59 as it is complemented by a water molecule that allows to maintain the pentagonal H-bond network. Thermodynamic measurements by differential scanning calorimetry and circular dichroism show that the complexes with pVIPR exhibit higher stability than the pLMP2 complexes, irrespective of HLA-B27 subtype. The results indicate that HC polymorphism can influence the conformation of the presented peptides, with obvious consequences for T cell recognition.

A11

Incompatibility between KIR2DL and Its HLA-C Ligands Is Associated with Acute Rejection in Kidney Transplantation

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Aim: Killer-cell-immunoglobulin-like receptors (KIRs) are expressed by natural killer (NK) cells and subsets of T cells. Lately, it has been shown that KIR/HLA incompatibility exerts beneficial effects in bone marrow transplantation. Despite the knowledge about recipient NK-cell cytotoxicity against the graft in renal transplantation, little is known about the functional role of KIR/Ligand incompatibility in this transplant setting. **Methods:** We designed a PCR-SSP for genotyping of 16 KIR genes amplifying gene products ranging from 107-455bp. Genotyping for HLA was

performed routinely prior to Tx. In our study 42/94 patients suffered from one or more biopsy proven acute rejection (aRx) episode after kidney transplantation. **Results:** Data were analyzed for every single KIR/HLA ligand pair on the current knowledge of HLA binding for aRx patients compared to the control group. In summary, patients with stable renal function displayed a significant higher number of matches between the inhibitory receptor KIR2DL1 and its corresponding HLA-C group 2 alleles ($p<0,05$, Pearson´s chi-squared test) compared to the aRx group. **Conclusion:** These are the first data illustrating the influence of KIR/HLA incompatibility in renal transplantation suggesting that KIR/HLA incompatibility might lead to an imbalance between inhibitory and stimulatory signals. We suggest that allografts which do not express recipient MHC class I molecules, can be potential targets for NK-cell killing influencing graft outcome.

A12

The Donor Cell Source Is Important for the Evaluation of Crossmatch Results with Sera Containing HLA Class II Antibodies

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The aim of the study was to investigate the influence of the donor cell source on the complement-dependent cytotoxicity crossmatch (CDC-XM) with sera containing HLA-class II antibodies (Abs).
Study design: Un-separated peripheral blood lymphocytes (PBLs), separated T- and B-lymphocytes, and spleen-cells from 12 different donors were analysed by CDC-XM against 13 HLA-class I+II and 9 HLA-class II Ab positive, blood group compatible sera (n=202). The number of crossmatches given positive according to the laboratory protocol and the p-value comparing PBL versus B- lymphocytes and versus spleen-cells were evaluated.
Results: A significant increase in positive CDC-XM results performed with spleen-cells (108/202) were observed compared to PBLs (48/202), for sera containing HLA-class I+II Abs, as well as for sera solely positive for HLA-class II Abs. Separated B-lymphocytes (120/202), as an alternative cell source, showed equivalent results compared to spleen-cells in both sera groups. More often positive CDC-XM results were found in separated T-lymphocytes (74/202) compared to PBLs.
Conclusion: Within the Eurotransplant standards, the preferred cell source for performing a pre-transplant-XM was changed from spleen-cells to PBL in order to reduce the cold ischemia time. However, reliable CDC-XMs with HLA-class II-Ab positive sera might only be achieved using B-lymphocytes or spleen-cells as targets.

A13

Inducible Knockdown of HLA Expression

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HLA polymorphism is the most relevant barrier to the development of cell-based therapies for regenerative purposes. To overcome this limitation, we used RNA interference (RNAi) to specifically knock down HLA class I transcripts. Regions susceptible to the action of small interfering RNAs (siRNAs) were identified in HLA-A heavy chain and beta-2-microglobulin (beta-2m) transcripts to achieve a gene or class I specific HLA silencing, respectively. Lentiviral vectors were designed to express short hairpin RNA sequences (shRNA) targeting HLA-A heavy chain or beta-2m constitutively or controlled by a doxycycline-inducible promoter system. The level of HLA suppression in HeLa cells and B-LCLs was detected by flow cytometry, real-time RT-PCR and Western Blot. Complement- dependent cytotoxicity assays were performed to evaluate the protective effect of HLA suppression against immune response. Hela cells stably transduced with lentivirus expressing shRNA showed a reduction of 85% in HLA-A and 50% in beta-2m. The transduction of inducible RNAi cassettes containing the sequences for shRNAs targeting beta-2m suppressed HLA class I expression up to 50% in HeLa or 60% in B-LCLs in a fully reversible manner. In a complement-mediated cytotoxicity assay it was demonstrated that HLA knockdown was very effective in preventing antibody-mediated cell lysis. In conclusion, we demonstrated the feasibility of controlling HLA expression by genetically modifying cell-based therapeutics to overcome the limitations of immunological rejection, bringing cellular therapies closer to reality.

A14

The First HLA-DRB1 NULL Allele: DRB1*0710N Is the Result of a Frame-Shift Causing Deletion

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Of the 54 HLA null variants that have been described to date in the IMGT database, only five null alleles are in DRB genes, all of which in DRB4 and DRB5. So far, no DRB1 allele with absent expression at the cell surface has been described. We here describe the identification of the first such allele, HLA-DRB1*0710N, which has been detected in a male Caucasian potential bone marrow donor from the North German Bone Marrow Registry (NKR). The complete HLA typing of this donor was A*01, *02; B*18, *44; DRB1*0710N, *11; DQB1*02, *03. Cycle sequencing of exon 2 for DRB1 was performed following haplotype-specific amplification using an Applied Biosystems 3730 sequencer, resulting in the detection of the new allele. This new allele is identical to DRB1*0701 and DRB1*0709 except for a deletion of two consecutive nucleotides at positions 175 and 176 in exon 2, causing a frame shift which results in a premature stop codon at codon 32. The data has been submitted to the IMGT/HLA database and the name DRB1*0710N has been officially assigned by the WHO Nomenclature Committee in May 2005. The nucleotide sequence is available in the EMBL, GenBank and DDBJ Nucleotide Sequence Databases under the accession number AJ968418.

A15

Duplication of HLA-B and HLA-C Genes in Two Unrelated Caucasian Families.

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Typing of voluntary unrelated blood stem cell donors identified two unrelated Caucasian families with additional HLA-B and -C genes.
Serological and PCR-SSO typing did not allow an unambiguous assignment of HLA-B and -C antigens/alleles: three HLA-B antigens were detected based on the serological reaction pattern; additional positive reacting probes were detected by PCR-SSO. The existence of extra HLA-B and -C alleles was confirmed by sequence based typing. The HLA haplotypes carrying the additional alleles were: HLA-A*03-B*07-B*13-Cw*06-Cw*07-DRB1*15-DQB1*06 and HLA-A*23-B*44-B*49-Cw*05-Cw*07-DRB1*13-DQB1*06.
HLA typing of available family members in three generation was performed in both families. The additional HLA-B and -C alleles were found to be inherited over several generations. Based on the proper inheritance of the alleles a chromosomal aberration could excluded. Since the doubled genes are inherited in linkage in all cases, the additional genes may be located quite close to each other on the MHC.
The mechanism of the HLA-B and -C duplications probably may have worked through unequal crossing over. There are no other reports in the literature, describing the existence of additional HLA genes, but there are reports about the loss of HLA loci. Since unequal crossing over can lead not only to the duplication of genes, but also to gene losses, both phenomena may be caused by the same mechanism. In our case of HLA-B and -C duplications the molecular mechanism can only be elucidated if the doubled genes can be exactly located, by sequencing the HLA-B and -C loci and by microsatellite analysis.

A16

The Novel Immunosuppressant Sanglifehrin A Shows a Potent Inhibition of Interleukin-6 Production in Both T-Lymphocytes and Monocytes

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The novel immunosuppressant Sanglifehrin A (SFA) is an immunophilin-binding metabolite with a yet unidentified mechanism of action. Several reports demonstrated the effects of SFA on proliferation and cytokine production of purified T-cells with varying results. However, less is known about its impact on the regulation of innate immune responses.
A whole-blood-assay was used to investigate the impact of SFA on monocyte and T-lymphocyte activity/proliferation upon LPS and Anti-CD3/Anti-CD28 stimulation, respectively.
SFA inhibited proliferation, CD25 expression and IL-2 protein expression of T-lymphoctes. Whereas IL-2 mRNA expression was significantly reduced after 4 hours, the mRNA expression of IL-4, IL-6 but not TNF-α were inhibited both after 4 and 24 hours of costimulation. The production of IL-2 and IL-6 protein in T-lymphocytes was even stronger affected than the mRNA expression of the respective cytokine. Unlike other immunophilin-binding immunosuppressants, SFA also inhibited LPS-induced IL-6, TNF-α mRNA and protein expression as well as intracellular IL-6 production in CD14+ monocytes.
We propose that SFA may have a significant effect on the initiation and direction of immune responses. Considering the pleiotropic role of IL-6 at the interface of innate and acquired immunity, these novel aspects of the unique immunosuppressive action could strongly impact on future clinical applications of SFA.

A17

Genetic Associations of Primary Autoimmune Neutropenia

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Purpose: The aetiology of primary autoimmune neutropenia is not known. However, frequent formation of HNA-1a specific autoantibodies suggests a genetic predisposition. In fact, autoimmunization against the neutrophil specific antigen HNA-1a (NA1) was reported to be associated with HLA-DR2. However, HLA class II typing was performed serologically and only a low number (N=26) of affected infants was tested. In addition, this work was restricted to HNA-1a specific autoimmunization.
Methods: We analysed 116 neutropenic infants (< 3 years) with clinically and serologically confirmed primary autoimmune neutropenia irrespective of autoantibody specificity. We typed them for the FCGR3B (HNA-1), FCGR2A (H131/R131), FCGR3A (F176/V176) and HLA class II alleles using PCR-SSP methods. Statistic analysis was done by chi-square test with Bonferroni correction.
Results: As expected, the FCGR3B*01 (HNA-1a, NA1) allele was highly significantly associated with primary autoimmune neutropenia ($p<0,001$). Concerning the FCGR2A alleles, the H131 allotype was significantly associated ($p<0,05$). Unexpectedly, we found only a significant negative association with HLA DRB1*04 alleles primary autoimmune neutropenia ($p<0,05$). DRB1*01 alleles were markedly but not significantly more frequent.
The frequency of DRB1*14 alleles was increased threefold compared to the controls. Changes in frequencies of FCGR3A alleles were not significant. Our results confirm that the HNA-1a polymorphism of the Fc gamma receptor IIIb predisposes for autoantibody formation. This seems to be facilitated if the protective HLA DRB1*04 alleles are absent.

P: Poster

P18

KIR Introns-Polymorphisms, Frequencies, Disease Association

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KIRs recognize HLA class I ligands and can be found on natural killer cells and subsets of human lymphopoetic cells. 14 loci are clustered in a gene complex of about 200 kb.
The aim of this study is to investigate the variability of several intron sequences. Furthermore it is of interest, whether the intron alleles correlate with the susceptibility for autoimmune diseases, especially in combination with HLA class I molecules.
Genomic DNA from the members of ten Caucasoid families with two or more children as well as from patients with Celiac Disease or antiphospholipid antibodies and an ethnically matched panel was used for the amplification of the KIR introns 2 and 3. The following sequencing was performed by using the ABI BigDye terminator cycle sequencing chemistry on an ABI 3100 sequencer.
KIR2DL4 shows 7 polymorphic sites in Intron 2 with one of them being a minisatellite length polymorphism (3 or 4 repeats of a 34 bp fragment respectively). This results in the two different intron lengths of 866 bp or 900 bp. Seven different alleles were found. A length polymorphism also exists in intron 3 (dinucleotiderepeat GA 8,11,12) that has a length of 881 bp at most. Intron 2 of KIR3DL2 presents a length of 742 bp with 12 polymorphic sites and 8 alleles while intron 3 shows 10 polymorphic sites with 9 alleles in 1464 bp. A significant influence on disease susceptibility can not be observed by the polymorphisms tested for.
In conclusion we found very few polymorphic sites of the analysed KIR introns in contrast to other introns of the Ig-Superfamily. The highly conserved regions may be involved in functional processes.

P19

Gov (HPA-15)-Genotyping in Northern German Blood Donors

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Introduction: The Gov (HPA-15) system of the CD109 molecule, which is located primarily on platelets, activated T-cells and endothelial cells comprises the alloantigens Gov^a (HPA-15b) and Gov^b (HPA-15a), differing in an Tyr703Ser exchange. Gov alloantibodies are involved in platelet refractoriness, posttransfusion purpura (PTP) and cases of neonatal alloimmune thrombocytopenia.
Methods: PCR-ASP (PCR with allele specific primers) and PCR-PFLP were compared to establish a reliable method for genotyping of the Gov polymorphism. The best method was used to determine the allele frequencies within a cohort of German blood donors.
Results: In a first series, 109 samples were compared to each other in both assays showing six discrepant results with two false negative and one false positive reaction in each assay. Because PCR-RFLP was less clear to read and more time consuming, PCR-ASP was established as standard assay for genotyping. Up to now 132 samples have been screened with 18.9 % being $Gov^{a/a}$, 30.3 % $Gov^{b/b}$ and 50.8% being heterozygous. The calculated gene frequencies are 0.443 for Gov^a (HPA-15b) and 0.557 for Gov^b (HPA-15a).
Perspective: Gov genotyping of platelet donors will enable application of suitable platelet products in the case of refractoriness after platelet transfusion, neonatal alloimmune thrombocytopenia and after post transfusion purpura. The number of typed platelet donors will be consecutively extended. Platelets from typed donors can be used in the MAIPA assay for determination of platelet alloantibodies.

P20

No Association between CD45 Exon A Point Mutation (77C/G) and Idiopathic Dilated Cardiomyopathy (DCM) in German Patients

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Introduction: CD45 is a key molecule of the immune system expressed in various isoforms (ABC, AB, BC, B and 0), arising from cell type-specific alternative splicing of variable exons (A, B and C). A silent heterozygous point mutation (C to G transversion) at position 77 in exon A of the CD45 gene prevents splicing of exon A. Phenotypically, the transversion leads to permanent expression of isoform AB on activated T cells resulting in a variant CD45RA expression pattern. The CD45 77C/G polymorphism has been reported to be associated with the development of different kinds of autoimmune diseases. Since autoimmunity components are also postulated for the pathogenesis of idiopathic DCM, we performed a study to assess a possible association of the CD45 77C/G polymorphism with susceptibility for idiopathic DCM.
Patients and methods: We studied a total of 414 individuals (104 patients and 310 controls). CD45RA expression pattern on lymphocytes was examined by two colour flow cytometric analysis and subsequently the CD45 77C/G polymorphism was genotyped by polymerase chain reaction-allele specific restriction enzyme analysis.
Results: We found 5 patients and 8 control individuals displaying the variant CD45RA expression pattern. All identified individuals carried the heterozygous CD45 77C/G polymorphism. We did not find any homozygous individual carrying G at position 77 of exon A of the CD45 gene neither in the control group nor in patients group. The frequency of the 77G allele in the patient group was 2.4%, which did not differ significantly from 1.3% found in the control group ($p=0.327$).
Conclusions: Our data did not reveal any association between the CD45 77C/G polymorphism and susceptibility to idiopathic DCM in a German population.

P21

Identification of a Novel HLA-B*44 Variant (B*4441) in Three Unrelated Caucasian Individuals

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A new human leukocyte antigen-B*44 (HLA-B*44) sequence variant was identified by low-resolution routine genotyping of samples for a German bone marrow donor registry, the "Aktion Knochenmarkspende Bayern" (AKB). It was remarkable that this new allele was found almost simultaneous in three unrelated Caucasian individuals coming from the same rural area. All samples had been analyzed independently and had appeared noticeable in different PCR sequence-specific oligonucleotide (PCR-SSO) and PCR sequence-specific primer (PCR-SSP) tests. Sequence-based typing (SBT) using a mono-allelic technique confirmed the presence of a novel allele. The sequence differs from HLA-B*44020101 by two nucleotide positions at the beginning of exon 3: by position 353, at which a thymine is substituted by a cytosine, and by position 355, at which an adenine is substituted by a cytosine. These differences in sequence result in deviant amino acids at codon 94 (Ile94Thr) and codon 95 (Ile95Leu). In this section the primary structure of the new B*44 allele officially designated as B*4441 is identical to the HLA-B*070201 consensus sequence. The intron 2 is a regular B*44 one. It is therefore probable that the new allele originated by gene conversion involving an allele containing the consensus pattern at the beginning of exon 3.

P22

A Haplotype DRB1*13 – DQB1*06 with a New DQ Allelic Variant Closely Related to DQB1*0618

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HLA-molecules are characterised by a high polymorphism. Complex HLA typing techniques are therefore required to achieve a high level of histocompatibility between donor and recipient. In the present report, we described a novel HLA-DQB1 allele, that has been observed in a BSC donor from our local registry.
High resolution DQB1 typing was performed using onelambda allele Specific HLA Class II (DQB1) SSP and the HLA AlleleSEQR-DQB1 Typing Kit by Atria Genetics. Typing by sequence specific primer (SSP) amplification revealed a novel pattern similar to DQB1*0202 by with further undefined PCR bands that did not match any of the criteria for the known DQB1 alleles. Direct sequence analysis revealed a novel heterozygous position at nt 184 in exon 2 of the DQB1 gene. Both forward and reverse sequence on two separate blood sample of the donor confirmed this heterozygous pattern. To obtain further information on the genotype the parents of our donor were asked to send us some buccal swabs for further HLA typing. Results of the mother were DQB1*0501,*0202, results of the father were DQB1*0503,*06XX. Family based genotype analysis defined that the novel DQB1 allele belongs to the haplotype HLA-A*01, B*1517, DRB1*13 DQB1*06XX. The DQB1*06XX allele is closely related to DQB1*0618. The difference between DQB1*0618 and the new allele is the point mutation at nt 184, leading to an amino acid exchange from thyrosin to histidin.
Conclusion: The sequence of the novel HLA-DQB1*06xx has been reported to the EMBL database and to the IMGL nomenclature commission.

P23

A New Recombination Product of an DRB3* Allele and the DRB1*1302 Allele: HLA-DRB1*1367

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On the occasion of regular HLA-typing of a female potential stem cell donor with SSP and SSOP, we found inconclusive amplification patterns even with different HLA typing sets for DRB1 alleles. PCR-based class I and class II typing revealed the HLA pattern A*0201, *0101; B*4001, *5701; DRB1*13, *0701; DRB3*0301; DRB4*0103N, DQB1*0604, *0303; DQA1*0102, *0201. We found a new DRB1*13 allele, similar to DRB1*1302. When we sequenced the DRB1*13 with the corresponding ABI exon primer based SBT (Sequencing Based HLA Typing) kit we found an regular DRB1*1302 allele, but when we used the corresponding protrans kit (intron primer based SBT), we found an extraordinary DRB1*1302 variant.
This new variant should be a recombination product of an DRB3* allele (DRB3*0301?) and the DRB1*1302 allele. The first part of exon 2, which is the binding site for group specific amplification primers, belongs to the DRB3* group motif (codons: 9-14). This DRB3* group motif is also found in the DRB1*1130 allele. After submission of the DNA sequence (exon 2) to the EMBL/GenBank/DDBJ-Data Banks and to the WHO Nomenclature Committee for Factors of the HLA System, the new allele was named HLA-DRB1*1367 (HWS10002707, Accession number: AJ853708). In relatives and siblings of the potential stem cell donor we found the DRB1*1367 allele in 2 of her 3 children. Only SBT with intron based amplification and sequencing primers gave the correct result. This is an example that exon primer based SBT only may not be sufficient in certain cases.

P24

Low Alloreactive Potential of HLA-B*0739 when Mismatched with HLA-B*0702

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We here report the identification of a new HLA-B*07 allele in a male Caucasian. This new allele was initially typed as B*0713 by sequence- pecific primed PCR. Because of the infrequence of that allele, a sequencing-based typing was performed to confirm that result. This yielded the detection of the novel allele HLA-B*0739. It is actually closest to B*070201, while it differs from B*0713 in 12 positions in exon 2. The nucleotide sequence is available in the EMBL, GenBank and DDBJ Nucleotide Sequence Database under the accession number AJ870971. Compared to B*070201, the new variant is characterized by a nonsynonymous nucleotide exchange (C->T) at nucleotide position 118 of exon 2. Previously this was considered a constant position, suggesting that it is likely to be caused by a single point mutation. It results in the amino acid exchange Ala->Val at position 40 of the mature polypeptide. Since this position is located in an outer loop of the HLA molecule, it is highly unlikely to affect peptide binding or T-cell receptor interaction. Thus, the newly found allele should have a low alloreactive potential in case of a mismatch to the most common HLA-B allele B*0702.

P25

Position 47 Serves as a Keystone Position in HLA-DRB1 and Is Affected in HLA-DRB1*1211

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Currently 10 different amino acid variants of the HLA-DRB1*12 family are known. We here report the identification of a new HLA-DRB1*12 allele in a healthy Caucasian male individual. The allele was detected by sequencing-based typing during confirmatory high resolution typing of a male potential unrelated donor from the Czech National Marrow Donors Registry. Compared to DRB1*120101, to which it is closest, the new variant is characterized by a new replacement mutation (T->C) at nucleotide position 126 of exon 2, resulting in the amino acid substitution Phe->Leu at position 47. This residue is part of pocket E of the peptide binding groove and is thus directly involved in peptide binding. The new allele, DRB1*1211, is therefore likely to differ substantially in its peptide binding repertoire and alloreactive potential from other DRB1*12 alleles. In addition, computational analysis, which utilized crystallographic data, revealed position 47,s keystone functionality in the ß-1 domain, joining both segments of the alpha helix with the beta sheet and that this position plays a major role in the structural conformation of the binding groove. As this mutation results in an essential protein function impairment by computational modeling, position 47 should be considered as a keystone position. As the identification of keystones allows for weighting of individual residues, this is extremely valuable for matching algorithms and peptide binding predictions.

P26

Confirmation of A*020109 Using HaploPrep™ for HLA-A

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During routine HLA typing by PCR-SSP using HLA A* low resolution typing kit (Olerup SSP AB, Sweden), a caucasian patient and her identical brother were typed to be A*01; in contrast to serological typing (Biotest, Germany) which determined A1,2; for both. Subsequent high resolution typing with the Olerup SSPTM HLA-A*02 typing kit confirmed the serological A2 as an A*0201 allele. The result of sequence based typing (Applied Biosystems, Foster City,CA, USA) was A*010101,*020109.
The rare allele A*020109 was first found in a Caucasian typed at the Johns Hopkins University, USA, was submitted to the EMBL/GenBank/DDBJ database in Oct. 2002 and is assigned in the IMGT/HLA database under the accession number AY158885.
To confirm the A*020109 allele we separated sample DNA into its haploid components by HSE (Haplotype-Specific Extraction) with HaploPrepTMpending using probe A299T on the GenoM6. Probe A299T covers all A*02 allels except of A*020602 but no A*01 allels.
Subsequent sequence based typing of the segregated allel was done using the HLA-A High Resolution Typing System (Abbott, USA). This test uses a generic primer pair for the amplification of HLA A exon 2, 3 and 4 and forward and reverse sequencing primer for each exon.
The allel assignment was performed with the Match Tools™ and the MT Navigator™ software (PE Applied Biosystems) and showed the A*020109 allel which differs from the common A*020101 in exon 2 at base position 144. The sequence was submitted to EMBL Nucleotide Sequence Database under the accession number AM040715.

P27

Luminex-Labtype und PCR-SSP: Ein Vergleich

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Problem: Ist die Luminex-LABType-Technik die Ablösung anderer mokularbiologischer Methoden oder des CDC bzw. schafft sie, was die andereren DNA-Techniken nicht schaffen?
Material/Methoden: 600 HLA-Typisierungen (562 Routine- und 38 Qualitätskontrollproben: 18 UCLA, je 10 Eurotransplant und Instand-Düsseldorf) wurden mit PCR-SSP (Genovi--sion, Wien/Österreich) und mit der Luminex-LABType-rSSO Microbeadstechnik (One Lambda, Inc., Canoga Park, CA, USA) entsprechend den Manuals der Hersteller durchge-führt. Die PCR-Produkte von HLA-A*, -B*, -Cw*, -DRB1*, -DQB1* wurden elektrophoretisch geprüft.
Resultate: Der Vergleich der 2-digit-HLA-Typisierungen zwischen LABType und PCR-SSP ergab je 2 Differenzen bei HLA-A bzw. HLA-B unter 18 UCLA-Proben, 0 Differenzen unter 10 Eurotransplantproben, 0 Differenzen unter 10 INSTAND-Düsseldorf-Proben und 6 HLA-A- und 5 HLA-B-Differenzen unter 562 Routine-Proben. Differenzen zwischen der LABType und der PCR-SSP bei 4-Digits-Auflösung waren: A*0246,2408 / A*0201,0206; A*0246,2408 / A*02012402; A*no result / A*0201,2601; A*0246,2408 / A*0201,0206; A*0101, 80XX / A*0101,0103; A*2407,2410 / A*2407,bl.; B*4040, 07XX / B*4002,0702; B*1814,35XX / B*1801, 3505; B*46XX,4006 / B*1802,3505; B*1501,35XX / B*1501,1335; B*1501,57XX / B*1501,1503; B*35XX,81XX / B*3501,3503.
Fazit: Die relativ leichte Test-durchführung, sichere Resultate sowie ein annehm barer Preis sprechen für LABType-Luminex in 2-Digits-Auflösung. Die 4-Digits-Auflösung

ist vor Benutzung in Kombination mit etablierten Techniken des jeweiligen Users zu prüfen. Diese Methode ist günstig bei permanentem Missverhältnis zwischen Laborbesetzung und anfallender Steigerung der Probenzahl bzw. bei generell hohem Probendurchsatz, jedoch nicht für die akut anfallende Einzelprobentypisierung.

P28

The Mobile Immunological Communicator for Timely and Precise Decision Making

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Beside the quality of HLA typing and crossmatching a list of administrative factors are critical for the function and survival of transplanted organs. These concern the safety of data transmission to the immunologist which is usually done by phone. In order to address this issue effectively the mobile communication unit 'Mobile Immunological Communicator' (MIC) was developed. MIC offers two seperate modules. The MICBoard, which is implemented to a mobile device (e.g. to a cell phone), representing the user interface and the MICServer providing an application service for data processing. The user is requested to input the ET number of the recipient, the ET number of the donor and the donor,s HLA-typing. These data are sent by the MICBoard to the MICServer. The MICServer provides the complete data of all patients with status 'transplantable'. The recipient,s ET number will be linked with the most current data. The MICServer performs the HLA matching and identifies whether forbidden antigens are known for the recipient. The histocompatibility between donor and recipient is then examined by taking into account the forbidden antigens as well as crossreactivities. The result of this calculation and the actual data of the recipient are transmitted to the user through the MICBoard. The MIC ensures a reliable exchange of information on a high safety level. Thus, a reduction of the time required for decison making as well as a substantial increase of the decision quality is achieved.

P29

Conceptual Pathway for a Data Management System in Blood Stem Donor Registries

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Data management is of considerable importance in recruiting and testing large numbers of voluntary blood stem cell donors. These donors are registered including a basic HLA pattern used to identify potential recipients. We have established a barcode based data management system in order to simplify and safegard this process.
Donor registration, documentation and data archives were changed from a manual system to a donor registration form containing a barcode registration number, barcode labels for sample identification and a donor ID-card. Incoming samples and donor registration forms are processed using a sample registration software that controls for missing material or forms. The data is further processed to a laboratory information management. The donor registration forms are digitally archived and archived donor numbers can be used by the LIMS to create test request and working lists. All donor samples are DNA extracted using the fully automated Biorobot MDX (QIAGEN, Hilden, Germany). The basic HLA registration typing is performed using high-troughput hybridisation. After analysis and validation, donor results are transferred by interfaces in the LIMS. Result files can be generated by the LIMS for import into the DoCom software.
Using the data management experience from blood transfusion processes, we have established a barcode based handling procedure for HLA-typing of large samples of blood stem cell donors. By using interfaces between the software for digital archiving, sample registration, and laboratory test systems clerical errors can be reduced and hand-on time can be saved.

P30

The Modular Concept for Peptide Prediction Allows for Computationally Managing Genetic Diversity to Find Personalized GvL-Ligands

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A variety of algorithms have been successful in predicting HLA-peptide binding for HLA variants, for which plentiful experimental binding data exists. While predicting binding for only the most common HLA variants may provide sufficient population coverage for vaccine design, successful prediction for as many HLA variants as possible is necessary to understand the immune response in transplantation. The high cost of peptide sequencing limits the acquisition of binding data. A prediction algorithm, which applies the binding information from well studied HLA variants to variants, for which no peptide data exist, is necessary.We have developed a modular concept of HLA-peptide binding prediction. In a crosschecking process of the new algorithm highly accurate predictions were made for all characterized alleles without using their experimental peptide binding data. Using the MHCBN peptide database, and a minimum cutoff of 15 peptides, the modular concept increased the number of predictable alleles from 15 (4.5%) to 75 (22.3%) of HLA-A and 12 (2.0%) to 36 (5.9%) of HLA-B proteins. Under the modular concept, binding data of certain HLA molecules can make prediction possible for numerous additional HLA alleles. In this regard, the HLA molecules A*7401, 3201, 6813; B*1803, 4103, 3908 have been identified to be the most informative. Achieving peptide binding prediction for all HLA molecules will provide a major basis for computionally managing genetic diversity to find personalized GvL-ligands and to individualize the clinical application of the minor antigen concept for the management of GvL reactions.

P31

Requirement of Helper Effect on Induction of HA-1H Cytotoxic T Cells from Naive Precursors

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The efficacy and specificity of cellular immunotherapy against hemtologic malignancies may be improved by in vitro generation and expansion of leukemia reactive T cells. Since expression of minor histocompatibility antigen HA-1H is limited to hematopoietic cells, ex vivo generated HA-1H-specific cytotoxic T cells (CTLs) could be applied for adoptive immunotherapy. Primary CTL induction from naïve precursors requires professional antigen-presenting cells. Here we demonstrate the feasibility of ex vivo induction of HA-1H-specific CTLs when CD4+ T-cell help was provided during primary stimulation. As a stimulus for the induction of T-cell help we used tetanus toxoid (TT), known to be presented via HLA class II. PBMCs of HLA-A*0201 positive and HA-1H negative donors were stimulated with TT and HA-1H peptide. Cells were restimulated weekly with irradiated peptide-pulsed PBMCs. HA-1-specific CTLs were visualised by pentameric HLA-A*0201/HA-1 complexes. After second restimulation cycle 1.1% of CD8+ cells responded to the HA-1H. CTLs were expanded in culture flasks coated with anti-human CD3 and CD28 mAbs. Cells expanded for 14 days were 87% HA-1H positive and showed the classical phenotype for CD8+ effectors represented by high expression of CD45RO and HLA-DR. The lymphokine production of TT-activated CD4+ lymphocytes is critical for the induction of naïve CD8+ precursors. We here report a fast, cost effective and efficient protocol that can easily be adapted to GMP conditions for translational purposes.

P32

Expression der extrazellulären Domänen eines kreuzreaktiven T-Zellrezeptors

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Morbus Bechterew ist eine sehr stark mit HLA-B27 assoziierte Autoimmunerkrankung. Verschiedene Subtypen (z.B. B*2705 / B*2709) zeigen eine differentielle Assoziation, obwohl sie sich lediglich durch eine einzige Aminosäure in der Peptidbindungsgrube unterscheiden. Röntgenkristallographische Untersuchungen (*J.Biol.Chem.*280: 2962-2971; 2005) an B*2705 und B*2709 im Komplex mit einem viralen (pLMP2) und einem Selbst-Peptid (pVIPR) zeigten, daß die Peptide subtypabhängig in drastisch differenten Konformationen präsentiert werden. Molekulare Mimikry tritt hierbei nur bei dem krankheitsassoziierten Subtyp B*2705 auf. Weiterhin konnten kreuzreaktive zytotoxische T-Zellen gegen beide B*2705:Peptid-Komplexe identifiziert werden. Bisher sind keine Strukturen von HLA-B27 / T-Zellrezeptor-Komplexen bekannt. Um aber einen tatsächlichen Beweis für molekulare Mimikry zu erhalten müssen die Bindungseigenschaften und die Struktur der α- und β-Kette eines kreuzreaktiven T-Zellrezeptors (TCR) ermittelt werden. Unser Ziel besteht deswegen darin, Komplexe eines kreuzreaktiven TCR mit ausgewählten HLA-B27 Molekülen röntgenkristallographisch zu untersuchen. Hierfür wurden zunächst die extrazellulären Domänen des MPVPAC7-TCR in *E.coli* exprimiert. Experimente zur Rekonstitution funktioneller TCR und zur Komplexierung mit B*2705-Molekülen werden z.Z. durchgeführt.

P33

Mixed Chimerism within Peripheral Blood, Bone Marrow and Purified Leukocyte Subsets after Allogeneic Blood Stem Cell Transplantation

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Purpose and Methods: Chimerism analysis has become a routine diagnostic procedure after hematopoietic allogeneic stem cell transplantation (HSCT) for early detection of relapse of disease or graft failure. Whereas some centres developed individual in-house short tandem repeat (STR) systems, others prefer commercial multiplex PCR systems. However, little is known about inter-assay variation, which could have a significant impact on treatment decision. We therefore compared two commercial multiplex PCR kits [AmpFLSTR® Profiler® and AmpFLSTR® COfiler® PCR Amplification Kit (Applied Biosystems/ABI/; Weiterstadt, Germany)] with our individual in-house STR system. The comparison was performed by testing STR markers in peripheral blood (PB), bone marrow (BM) and specific leukocyte subsets.
Results: A total of 36 samples of four pediatric patients were analyzed by both in-house and commercial STR systems. When examining PB and BM, we did not observe significant differences. In addition, no differences were seen when purified leukocyte subtypes were investigated. To survey the validity of individual STR primers, several markers which are components of the in-house and of the commercial STR system did not reveal differences.
Conclusion: Our study clearly shows that even in purified leukocyte subsets similar results can be obtained using different STR-PCR methods. This may help to increase interlaboratory standardization of chimerism analyses for early clinical intervention.

P34

HLA-Antikörper-Screening mittels LUMINEX Technologie - Erfahrungen aus dreijährigem Einsatz in der Laborroutine

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Zum Nachweis von HLA-Antikörpern ist der Lymphozytotoxizitätstest (LZT) seit vielen Jahren im Rahmen der Transplantation als Standardmethode etabliert. Seit einiger Zeit werden jedoch vermehrt sensitive ELISA oder Durchflusszytometer basierende Tests zum HLA-Antikörper-Screening eingesetzt, wobei die Relevanz der durch neue Verfahren ermittelten HLA-Spezifitäten noch nicht geklärt ist.
In dieser Studie haben wir die Ergebnisse aus drei Jahren HLA-Antikörper-Screening mittels Luminex Verfahren (LABScreen, One Lambda Inc.) zusammengefasst und mit etablierten Testverfahren (LZT und ELISA) verglichen.
Wir untersuchten 485 Seren von Patienten der lokalen Wartelisten auf HLA-Kl. I sowie 347 Seren auf HLA-Kl. II Antikörper. In 241 (50%) der Seren konnten mittels LZT und LABScreen Methode HLA-Kl. I Antikörper spezifiziert werden, allerdings nur in 121 Seren mit identischen Spezifitäten. Die LABScreen Methode ergab in den verbliebenen Seren zusätzliche Spezifitäten (7 Seren mit zusätzlichen ≥splits„ innerhalb des gleichen ≥broad„ Antigens, 72 mit zusätzlichen kreuzreaktiven (CREG) Spezifitäten, 104 mit zusätzlichen differenten Spezifitäten) sowie 46 Seren mit komplett differenten Resultaten der beiden Testverfahren.
Unsere vergleichende Analyse zeigte, dass insbesondere problematische Seren mit sowohl sehr hohen als auch niedrigen Antikörper-Titern mit den neuen Verfahren besser charakterisiert werden konnten. Allerdings erwies sich die Interpretation der durch die Software ermittelten Ergebnisse häufig als problematisch. Auch der Einfluss von kreuzreaktiven HLA-Spezifitäten auf die endgültige Akzeptanz eines möglichen Organspenders bleibt zu diskutieren.

P35

Screening and Crossmatching for HLA Antibodies Prior to Allogeneic Stem Cell Transplantation

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The presence of patients` anti-donor alloantibodies can increase the risk of graft rejection in allogeneic stem cell transplantations, and a positive crossmatch against donor lymphocytes may be a predictor for graft failure.
We studied 28 patients who were transplanted with allogeneic stem cells in our centre. All patients were screened for HLA class I and class II antibodies by ELISA based methods at the time of registration, prior to and in monthly intervals after transplantation. Donors also were tested for HLA antibodies. CDC based B- and T-cell crossmatches were done prior to transplantation in 18 cases, depending on the availability of viable donor cells.
One donor and two patients had anti HLA antibodies before transplantation, which weren`t directed against transplanted antigens, as these transplantations were fully matched. All the pretransplant crossmatches turned out negative. Post transplant screenings for patients` HLA antibodies were done between day +8 and day +148 with a mean observation period of 53 days. Two patients developed HLA antibodies after transplantation, which weren`t directed against donor antigens eighter. No graft rejection was reported on HLA antibody positive patients.
Among our patients HLA antibodies didn`t raise a problem in transplantation schedule. Nevertheless we routinely evaluate all patients for HLA antibodies whenever a donor search is started and screen all patients and donors for antibodies against non shared HLA antigens prior to transplantation as donor cells of sufficient viability for crossmatching aren`t always available.

P36

Follow-Up of Unrelated Allogeneic Stem Cell Donors – First Results

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To promote PBSC donation among healthy unrelated volunteer donors data are to be collected to establish the long-term safety of G-CSF stimulation. From 2000 to 2004 16 PBSC aphereseses on unrelated allogeneic donors have been carried out in our center. From 2003 on, all donors annually received a questionnaire concerning their actual state of health. 11 male and 2 female donors (81%) with an average age of 44,59 (29-56) years responded to the questionnaire. The mean observation period was 28,75 (3-53) months. 5 PBSC donors reported severe illness: One donor developed an exostosis of the 5th rib, one was operated on an umbilical hernia, one sufferes from recurrend articular and muscle pain accompanied by night sweat and weight loss, one has a chronic compensated renal failure, one has had diarrhoe and a common cold and suffered from fatigue and nausea. One female donor had recognized dizziness and an increased tendency for bruises as well as paraesthesia in boths arms. The disorders these donors reported occurred between 3 and 22 months after G-SCF stimulation. All the other PBSC donors have never been severely ill or under medical observation. No donor had fever of unclear origin, phlebitis, thrombosis or embolia, no donor recognized an increased tendency for epistaxis. Three donors need medication they didn`t have before G-CSF stimulation. One donor estimates that he is getting ill more easily than others, all the other donors feel to be in best physical and mental condition. No serious consequences clearly attributable to G-CSF stimulation have been identified among our donors. However, the number of donors enrolled is too small and the observation periods are too short to be conclusive.

M: MTA-Workshop

M1

Vierteljährlicher Serumversand

M. Bonn (MTA)

Transfusionsmedizin Freiburg, Germany

Wie sollten die beigefügte Unterlagen aussehen?
Welche Informationen müssen unbedingt enthalten sein!
Was kann das verschickende Labor tun um es möglichst einfach für dem Empfänger zu gestalten.
Vorstellung der Freiburger Versandliste und Bespiele.
Anschießend Diskussion mit möglichen Verbesserungen für alle ET-Labore.

M2

Kreuzprobe und Antikörper-Suchtest – der eine kann ohne den anderen nicht

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Mittels Vergleich unterschiedlicher HLA-Antikörper(Ak)-Testsysteme und ihrer spezifischen Erkennungspotentiale ist es möglich, ein exaktes Profil des humoralen Status, eines Transplantationskandidaten zu erstellen. Bei immunisierten Patienten und Patienten, die auf eine Re-Transplantation warten, ist eine erweiterte Ak-Suche mit mehreren Testsystemen von vorrangiger Bedeutung und beeinflusst die Entscheidung der Strategie bei der Kreuzprobe. Aufgrund seiner Definition ist das Ergebnis des Panel-reaktiven-Antiköprper (PRA) lediglich ein Annäherungswert;. wünschenswert ist prinzipiell die HLA-Spezifizierung des gegen fremdes HLA-Ag gerichteten Aks. Durch die Anmeldung in das ≥ Acceptable Mismatch Programm ist es möglich, hoch-immunisierten Patienten ein geeignetes Organ zu offerieren.
Ergebnis: Im Quartal IV/2004 waren 53/977 Patientenseren der Warteliste Nordbayern positiv für HLA-Klasse-I-Ak und 59/977 für HLA-Klasse-II. 27/59 der zweiten Serengruppe waren ausschließlich HLA-Klasse-II-positiv und nehmen derzeit nicht an der quartalsweisen Serumverschickung in andere Transplant-Labore teil. Die Durchführung einer erforderlichen B-Zell-Kreuzprobe bei HLA-Klasse-II-positiven Seren ist nur im Empfängerzentrum gegeben.
Zusammenfassung: Im Transplantationszentrum Nordbayern wird bei positiver B-Zell-Kreuzprobe von einer Transplantation abgeraten, d.h. bei Organangebot von einem externen Zentrum kann eine zusätzlich durchgeführte, positive B-Zell-Kreuzprobe zur Ablehnung des angebotenen Organs führten. Zur Diskussion steht, ob in allen

Transplantlaboren – bei zentrumseigenen wie auch bei verschickten HLA-Klasse-II-Ak-positiven Seren.

M3

HLA-Antikörpernachweis im ELISA-Based Single Antigen Assay (*LAT HD*)

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Bei Dialysepatienten sind HLA-Antikörper vor Nierentransplantation (NTX) mit einem erhöhten Transplantatversagen vergesellschaftet. Zum Nachweis dieser Antikörper ist der Lymphozytotoxizitätstest (LCT) seit langem etabliert. Zur Vereinheitlichung und besseren Reproduzierbarkeit der Ergebnisse wurden verschiedene ELISA-Tests entwickelt. Unterschiedliche Resultate dieser zwei Methoden gaben Anlass zu weiteren Untersuchungen.
Es wurden 373 für eine NTX gemeldete Patienten sowohl mit dem LCT als auch mit einem ELISA gemischter Klasse I-Antigene (*LAT-M™*, One Lambda, USA) untersucht. Davon reagierten 26 Patienten im *LAT-M* positiv, nicht aber im LCT. Außerdem waren 33 Patienten sowohl im *LAT-M* als auch im LCT positiv. Das Serum dieser 59 Patienten wurde mit einem Einzelantigentest (*LAT HD™*) nachuntersucht. In diesem Test können mit 59 verschiedenen Einzelantigenen der Klasse I HLA-Antikörper charakterisiert werden.

	LCT negativ (n=340)	LCT positiv (n=33)
LAT-M positiv (n=59)	26	33
LAT-M negativ (n=314)	314	0

Von 33 in beiden Tests positiven Patienten reagierten 32 auch im *LAT HD*. Die an EUROTRANSPLANT gemeldeten HLA-Antigen-spezifischen Antikörper bei 11 Patienten konnten in 10 Fällen im *LAT HD* nachgewiesen werden. Von den im LCT negativen Patienten reagierten 18 (69%) im *LAT HD* positiv. Es traten bei 88 Ansätzen durchschnittlich 11,7 (2-26) positive Reaktionen auf, die 8 (1-19) Antikörper anzeigten. Bei einem Patienten wurde ein Antikörper diagnostiziert, der zwei Jahre später im LCT bestätigt wurde.

	LCT negativ (n=26)	LCT positiv (n=33)
LAT HD positiv (n=50)	18	32
LAT HD negativ (n=9)	8	1

Der *LAT HD* kann als zusätzlicher Test zur Differenzierung von HLA-Antikörpern beitragen.

M4

A Novel ELISA-Based Crossmatch Procedure for Detection of Anti-HLA Class I and Anti-HLA Class II Antibodies

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The detection of donor-specific anti-HLA antibodies by standard procedures such as complement-derived cytotoxicity assays (CDC) is limited by its low sensitivity and the quality of the donor cells. Therefore, an ELISA-based technique was employed using solid-phase immobilized monoclonal antibodies to capture HLA class I or class II molecules of the donor, respectively. In this HLA class I or class II antibody monitoring system (AMS) the donor-specific anti-HLA antibodies from the sera of the recipients bind to the immobilized HLA-molecules of the donor. Upon the binding of donor-specific anti-HLA antibodies they are recognized by secondary enzyme-conjugated anti-human immunoglobulin (Ig) antibodies. A newly established modification of the standard protocol allows to differentiate between bound antibodies of the IgG and IgM isotype. Furthermore, this assay was adapted for investigating small amounts of solid tissue of donors from whom no other cells (e.g. from blood) were available. In the present cases of kidney and cornea transplantats and in a transfusion reaction the AMS-ELISA was shown to be a reliable tool to detect donor-specific anti-HLA antibodies which had not previously been identified by the classical CDC procedure. Therefore, the AMS-ELISA proofed to be a valuable tool for the post-transplantation monitoring of donor-specific anti-HLA antibodies during reaction crisis, after transfusion reactions and in particular cases of tissue transplantation lacking single cells.

M5

Vergleichende Untersuchungen der Teste ELPHA DRB lowRes bulk und ELPHA DRB1 dl

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Einleitung: Bei vergleichbarem Arbeitsaufwand (Einsatz von 3 Streifen im ELISA-Test) zwischen DRB lowRes und DRB1 dl kann ein 4 Digit-Ergebnis ohne DRB1* low durch DRB1 dl sowie die Klärung von Ambiguitäten erreicht werden. Bei dem Test DRB lowRes wird mit 24 Sonden gearbeitet, welche FITC markiert sind. Die Sonden sind auf 3 Streifen wie folgt verteilt: eine Sonde als positive Kontrolle, 20 Sonden für DRB1* und je eine Sonde für DRB3*, DRB4* und DRB5*. Die Bearbeitung erfolgt mittels ELISA-Technik (je eine Konjugat- und Substratphase, Reaktionsstop mit 1n Schwefelsäure, photometrische Messung im Gelblicht). In Auswertung dieses ELISA-Testes liegen die Allele des DRB1* Locus (2 Digit) in Assoziation zu DRB3*, DRB4* und DRB5* vor. Bei dem Test DRB1 dl wird mit 48 Sonden, verteilt auf 3 Streifen, gearbeitet. Davon sind 24 Sonden mit DIG und 24 mit FITC markiert. Die Bearbeitung erfolgt ebenfalls mittels ELISA-Technik, dabei gibt es aufgrund der Doppelmarkierung getrennte Konjugat- und Substratphasen für DIG und FITC. Zwischen den Reaktionen mit DIG und FITC erfolgt ein Inhibitorschritt (photometrische Messung im Blaulicht). Ziel dieses ELISA ist eine Auflösung für den DRB1* Locus bis zu 4 Digit. Um das Auflösungsvermögen der Teste zu beurteilen, sind folgende Proben parallel untersucht worden: 13 Proben mit bekannter 4-Digit-Auflösung (SSP high Resolution), 29 Proben mit einer bekannten Ambiguität im DRB1*-Locus (ELPHA DRB lowRes) und 61 Proben mit bekannter 2-Digit- Auflösung. Die Auswertung erfolgte mit der aktuellsten Biotest Software auf dem Level „häufige Allele“(Frequenz > 0,01%). Bei den Proben mit bekannter Auflösung von 4-Digit konnte 12 mal das 4 Digit Ergebnis bestätigt werden. Bei einer Probe (Ersttypisierung SSP-high Resolution DRB1*1305 und DRB1*0323) wurde jedoch auf dem Level „häufige Allele“ DRB1*1305 und DRB1*0301 gefunden. Hingegen wurde im Level „alle Allele“ auch DRB1*0323 mit angezeigt. Bei den Proben mit Ambiguitäten im DRB1* Locus konnten alle Ambiguitäten aufgelöst werden.88 der insgesamt 103 Proben zeigten ein eindeutiges Ergebnis mit 4 Digit im DRB1* Locus, bei 15 Proben wurden mehrere Ergebnisse angeboten. Dabei war der Unterschied nur im 3- und 4 Digit Bereich zu finden. Ambiguitäten traten mit dem DRB1 dl nicht auf.
Zusammenfassung: DRB1 dl erzielt in 85% der Fälle ein eindeutiges Ergebnis mit einer Auflösung von 4 Digit. Für eine Knochmarkspenderdatei ist somit bereits während der Ersttypisierung ein 4-Digit-Ergebnis im DRB1* Locus möglich.

M6

A Novel HLA Allele Found in a Slovenian Kidney Patient

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We describe a novel allele (HLA-B*27var) that was found in a patient waiting for kidney transplantation. Serological typing revealed an HLA-A3; B27; Cw2 phenotype, low resolution SSP typing an HLA-DRB1*15 genotype. While the HLA-A2 and Cw2 specificities were confirmed by low resolution SSP typing, the HLA-B DNA typing was not conclusive.
Sequencing of the HLA-B alleles was performed in two laboratories with two different protocols, commercial and home made. Assignment of alleles was performed by comparing the sequence to the latest release of the WHO nomenclature report. Both, exon 2 and exon 3 have been sequenced in both directions. Sequencing indicated that the patient was homozygous for a novel HLA-B allele, related to the HLA-B*27 group. When compared to the HLA-B*2702 sequence, HLA-B*27var has five nucleotide substitutions leading to 4 amino acid exchanges. The nucleotide substitutions are all located in exon 3 at positions 283 (C->T), 285 (C->A), 292 (A->G), 293 (T->G) and 299 (T->C), the amino acid exchanges are located at amino acid position 94 (Thr->Ile), 95 (Leu ->Ile) and 97 (Asn->Arg). While the exon 2 sequence of the novel allele is on basis of the so far known HLA-B*27 alleles identical with that of HLA-B*2702, the exon 3 sequence is identical with that of HLA-B*2719. However, on the basis of so far known amino acid motifs, a possible mechanism for the generation of this novel allele is a gene conversion event, where a HLA-B*2702 allele received a motif that occurs in many other HLA-B alleles, including most B*44 alleles. The novel allele was found also in the mother of the patient, who was heterozygous in her HLA-B alleles.

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Haplotypisierung von KIR-Genen

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Natürliche Killerzellen (NK) sind eine erste Verteidigungslinie bei der Abwehr fremder oder veränderter eigener Zellen. Sie tragen an ihrer Oberfläche Killerzell-Immunglobulinähnliche-Rezeptoren (KIR). Die Gene für diese KIR finden sich auf Chromosom 19q13.4 im Leukozytenrezeptorkomplex. Sie steuern Erkennung und Aktivierung von NK. NK-stimulierende Rezeptoren tragen einen kurzen zytoplasmatischen Anker (z.B. KIR2D**S**1), inhibierende hingegen einen langen zytoplasmatischen Anteil (z.B. KIR2D**L**4). Außerdem unterscheiden sich die KIR danach ob sie 2 oder 3 immunglobulinähnliche Domänen (D) besitzen. Analog zu DRB findet sich eine variable Anzahl KIR-Gene bei jedem von uns. Die drei KIR-Strukturgene (KIR3DL3, KIR2DL4, KIR3DL2) sind uns allen gemeinsam. Zwei Haplotypgruppen werden unterschieden: Haplotypen der Gruppe A haben nur ein stimulierendes KIR-Gen (KIR2DS4), während Haplotypen der Gruppe B 2 bis 7 stimulierende KIR-Gene haben können.
Es wurden mit einem PCR-SSP Test (Pel-Freez) 30 Knochenmarkspender und mit einem PCR-SSO Testkit (Luminex, OneLambda) 300 Knochenmarkspender für 16 KIR-Gene getestet. Die Haplotypisierung erfolgte mit dem Programm OLITYPE (Hum. Immunol. 1991 30:22-6) für die Haplotypen A und B1 bis B9. Neben den häufig vorkommenden KIR-Genotypen A,A und A,B1 wurden auch mehrere seltenere Varianten beobachtet.